

First Report of Pronamide-Resistant Annual Bluegrass (*Poa annua*)

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A biotype of annual bluegrass with suspected resistance to pronamide was collected from a golf course in Georgia. The objectives of this research were to determine the level of resistance to pronamide and the mechanisms associated with resistance. From POST applications, the pronamide rate that reduced shoot biomass 50% from the nontreated bluegrass measured >10 times higher for the resistant (R) biotype as compared with susceptible (S) biotypes. The R biotype was not controlled by PRE applications of dithiopyr or proflumicafene, but was controlled >92% by PRE applications of pronamide at 0.56 and 1.68 kg ha⁻¹. Mature plants (3- to 5-tiller) of the R biotype absorbed 32% less [¹⁴C]pronamide than the S biotype after 72 h in hydroponic culture and accumulated 39% less radioactivity per gram basis of dry shoot mass. The R biotype metabolized [¹⁴C]pronamide similar to the S biotype, averaging 16% of the extracted radioactivity. The resistance to POST pronamide applications in the R biotype is associated with reduced absorption and translocation compared with the S biotype.

Nomenclature: Pronamide (propyzamide); annual bluegrass, *Poa annua* L.

Key words: Absorption, mitotic inhibitor, translocation, turfgrass.

Annual bluegrass is a problematic weed in turfgrass. It has a light-green color, coarse leaf texture, and produces unsightly seed heads that reduce turfgrass aesthetics (Beard 1970). Annual bluegrass also has poor tolerance to heat, disease, and traffic stress that may further exacerbate reductions in turfgrass quality (Beard 1970; Lush 1989). The decline of annual bluegrass in late spring reduces turf density and predisposes areas to invasion of summer annual weeds. Practitioners can reduce populations by adjusting turfgrass fertility, modifying irrigation regimens, and collecting mower clippings after annual bluegrass seed head emergence (Gaussoin and Branham 1989; Lush 1989). Cultural practices that promote turfgrass competition with annual bluegrass often do not provide acceptable levels of control, and herbicide use may be warranted.

Mitotic inhibitors are widely used for PRE control of annual bluegrass in turf. The dinitroaniline (DNA) herbicides inhibit microtubule polymerization by binding to tubulin that restricts chromosome movement during prometaphase of mitosis and causes isodiametric cell formation (Blume et al. 2003; Murthy et al 1994; Vaughn and Lehnen 1991). Dithiopyr is a mitotic inhibitor used for PRE annual bluegrass control in turf that does not bind to tubulin. Rather, dithiopyr

binds to microtubule-associated proteins that shorten microtubules during mitosis (Armbruster et al. 1991; Vaughn and Lehnen 1991). Dithiopyr and the DNA herbicides have limited efficacy for POST annual bluegrass control. Application timing before germination is critical to maximize efficacy of these herbicides for PRE control (Bhowmik and Bingham 1990; Toler et al. 2003, 2007).

Pronamide (propyzamide) is a benzamide herbicide that provides PRE and POST annual bluegrass control in warm-season turfgrasses (Isgrigg et al. 2002; Johnson 1975; Willis et al. 2007). It arrests the prometaphase of mitosis, causing root-tip swelling in susceptible species (Vaughn and Lehnen 1991). Pronamide shortens microtubules located at the kinetochore region during mitosis, causing reformation of the nuclear envelope around chromosomes and polymorphic nuclei (Akashi et al. 1988; Vaughan and Vaughn 1987). The shortening of microtubules by pronamide is comparable to the symptomatology from dithiopyr on susceptible species (Vaughn and Lehnen 1991). Researchers have noted that pronamide controls DNA-resistant biotypes of annual bluegrass, goosegrass [*Eleusine indica* (L.) Gaertn.], and green foxtail [*Setaria viridis* (L.) Beauv.] similar to susceptible (S) biotypes (Delyé et al. 2004; Isgrigg et al. 2002; Vaughn et al. 1987). Therefore, pronamide may be an important tool for controlling annual bluegrass with resistance to DNA herbicides and other sites of action in turfgrass.

There have been 28 cases of herbicide-resistant annual bluegrass reported worldwide (Heap 2016). Resistance has been confirmed to ethofumesate,

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glyphosate, sulfonylureas, and triazines used for POST annual bluegrass control in turfgrass (Binkholder et al. 2011; Hutto et al. 2004; Kelly et al. 1999; McElroy et al. 2013). A common mechanism of herbicide resistance in annual bluegrass and other weeds is a mutated target-site enzyme or protein (Anthony et al. 1998; Cross et al. 2015; McElroy et al. 2013). Pronamide is the only mitotic inhibitor used in turfgrass that provides POST annual bluegrass control. Many turfgrass managers are using pronamide to control established annual bluegrass with resistance to other mechanisms of action. Resistance to pronamide has not been reported in annual bluegrass or from a turfgrass system.

An annual bluegrass biotype with suspected resistance to pronamide was identified on a golf course in Georgia. The superintendent had used multiple herbicides in rotation with pronamide for annual bluegrass control for more than a decade. The biotype was collected after segregation from susceptible annual bluegrass plants that were controlled from a POST pronamide treatment. The potential confirmation of pronamide resistance could have important implications for annual bluegrass control programs in turfgrass and other cropping systems. The objectives of this research were to evaluate the resistance levels of this biotype to pronamide and the mechanisms associated with resistance.

Materials and Methods

Plant Material. Five annual bluegrass plants were collected from the Willow Lake Golf Course in Metter, GA (32.42°N, 82.07°W) on April 24, 2015. These plants were uninjured from pronamide at a standard use rate, 1.68 kg ai ha⁻¹, applied approximately 3 mo prior to collection. The plants were transplanted to separate plastic pots with 79 cm² surface areas and 10 cm depths filled with sand and peat moss (85:15 v/v). Pots were placed in a greenhouse set for 23/17 C (day/night) at the University of Georgia Griffin Campus and irrigated to prevent moisture deficiencies. Seeds were collected by hand and immediately scattered over greenhouse flats filled with the aforementioned potting medium. Seed from two S biotypes was collected by hand and scattered over separate flats for establishment. These biotypes were collected in January 2015 from annual bluegrass populations indigenous to Griffin, GA (33.26°N, 84.28°W) and the Ryder Golf Course in Fort Bragg, NC (35.15°N, 79.0°W). The greenhouse flats were irrigated daily and fertigated

(MacroN 28-7-14 Sprayable Fertilizer, LESCO, Cleveland, OH) twice per week. Multileaf plants were transplanted to separate pots with 3.8 cm diameters and 20 cm depths filled with sand and peat moss. Plants were irrigated to prevent wilting, fertilized to promote growth, and allowed to reach a 3- to 5-tiller growth stage before treatments.

Dose–Response Experiments. The resistance levels of the suspected resistant (R) biotype were evaluated from rate titrations of pronamide. The R biotype was compared with the S biotype from Georgia in Experiment 1, and the S biotypes from Georgia and North Carolina in Experiment 2. Experiments 1 and 2 had three and two runs conducted, respectively. Treatments were applied in a spray chamber calibrated to deliver 187 L ha⁻¹ with a flat-fan nozzle (8002E, TeeJet Spraying Systems, Roswell, GA 30075). Pronamide (Kerb 3.3SC, Dow AgroSciences, Indianapolis, IN 46268) was applied at 0.03, 0.05, 0.1, 0.2, 0.4, 0.8, 1.7, 3.4, 6.7, or 13.4 kg ha⁻¹. This range was chosen so that the label use rates for annual bluegrass control in turfgrass (0.6 to 1.6 kg ha⁻¹) were in the midrange of rates evaluated (Dow AgroSciences 2013). Nontreated checks of each biotype were included. Plants were returned to the greenhouse at 1 h after treatment (HAT) and received ~0.6 cm of irrigation within 4 HAT. Irrigation was applied as needed to prevent plant wilting thereafter. Shoots were harvested at 4 wk after treatment (WAT) with shears. Biomass was oven-dried for 72 h at 60 C in Experiment 1, and then weighed. Fresh biomass was measured by weighing shoots immediately after harvest in Experiment 2.

Efficacy of PRE Pronamide Applications. Experiments were conducted to evaluate the efficacy of PRE pronamide applications for controlling the establishment of the R biotype, as compared with the S biotype from Georgia (delineated Experiments 3 and 4). Pots with 79 cm² surface areas and 10 cm depths were filled with the aforementioned sand and peat moss. Herbicides were applied to pots in the spray chamber before seeding of annual bluegrass. Treatments for Experiment 3 included indaziflam (Specticle Flo, Bayer Environmental Sciences, Research Triangle Park, NC 27709) at 0.05 kg ai ha⁻¹, prodiamine (Barricade 65WG, Syngenta Crop Protection, Greensboro, NC 27409) at 1.68 kg ha⁻¹, and pronamide at 0.56 or 1.68 kg ha⁻¹. A nontreated check was included. Treatments for Experiment 4 included dithiopyr at 0.42 kg ai ha⁻¹ (Dimension 2EW, Dow AgroSciences, Indianapolis, IN 46268),

prodiamine at 1.68 kg ha⁻¹, and pronamide at 0.56 or 1.68 kg ha⁻¹. The pots were returned to the greenhouse immediately after treatment. Seeds of the two biotypes were then sown over separate pots similar to previous research for evaluating PRE control (Judge et al. 2005; McCullough et al. 2013). Irrigation was applied as needed to prevent soil moisture deficiencies and promote seedling growth. Pots were fertigated weekly with the aforementioned methodology. Established plants were counted at 6 WAT in each pot. Two runs were conducted for both experiments.

Absorption and Translocation of Radioactivity following [¹⁴C]Pronamide Applications. Experiments were conducted to evaluate root absorption and translocation of [¹⁴C]pronamide in the suspected R biotype as compared with the S biotype from Georgia. Root uptake is necessary to control susceptible weeds with pronamide due to limited efficacy from foliar placements (Carlson et al. 1975). The current pronamide label for turfgrass also states that irrigation is essential after treatments to facilitate root uptake for weed control (Dow AgroSciences 2013).

Mature plants (3- to 5-tiller) of the R- and S biotypes from Georgia were removed from greenhouse pots, the roots were rinsed to remove soil, and the plants were grown hydroponically in an 11 L plastic tank filled with half-strength Hoagland solution (Hoagland and Arnon 1950). Twenty holes with 1.3 cm diameters, spaced 5 cm apart, were drilled in the lid of the tank. Roots from 10 plants of the two biotypes were placed through holes to allow suspension in the solution. The tank was then placed in a growth chamber (Percival Scientific, 505 Research Drive, Perry, IA 50220) set for 25/18 C (day/night) with a 12 h photoperiod of 350 μmol m⁻² s⁻¹. Two aquarium pumps were used to provide oxygen to the solution.

After 1 wk, tap water was added to the tank to bring the volume back to 11 L. Nutrient concentrations in the tap water were analyzed at the University of Georgia water-analysis laboratory. These tests found inconsequential nutrient levels (<1%) relative to the concentrations found in Hoagland solution, with the exception of calcium, which measured 6% of the recommended concentration (unpublished data). The tank was then spiked with a total of 92 kBq of [¹⁴C]pronamide (26 mCi mmol⁻¹, label position: U-benzamide ring; 98% radiochemical purity) plus 1 μM of technical-grade pronamide (Santa Cruz Biotechnology, Dallas, TX 75220). Plants were harvested at 72 HAT. Roots were rinsed

under a steady stream of tap water for 20 s and blotted dry with paper towels. Roots were then separated from shoots with shears, and samples were oven-dried for 7 d at 55 C. Samples were then combusted in a biological oxidizer (OX-500, R. J. Harvey Instrument, 11 Jane Street, Tappan, NY 10983), and radioactivity was quantified with liquid scintillation spectroscopy (Beckman LS 6500[®], Beckman Coulter, Fall River, MA 02720). Absorption was determined by dividing the radioactivity recovered by sample dry weight. Translocation was determined by dividing the ¹⁴C recovered in shoots by the total radioactivity in the plant (roots + shoots). Specific radioactivity in roots and shoots was calculated separately by dividing radioactivity in samples divided by dry weight.

Metabolism of [¹⁴C]Pronamide. The metabolism of pronamide was evaluated with a modified methodology from previous research with lettuce (*Lactuca sativa* L.), chicory (*Cichorium intybus* L.), and redroot pigweed (*Amaranthus retroflexus* L.) (Mersie et al. 1995; Rouchaud et al. 1987). Fifteen plants of the two biotypes (30 total plants) were grown hydroponically for 1 wk in the growth chamber using the aforementioned methodology. Plants were removed from the tank and placed individually in 5 ml plastic vials. The roots and shoots were positioned in the bottom and at the opening of the tubes, respectively. [¹⁴C]pronamide was applied to roots with a pipette at 5.8 kBq in 50 μl of tap water. Cotton balls were placed at the bases of shoots, and plants were returned to the growth chamber. At 2 HAT, 500-μl of tap water was added to vials to reduce moisture stress.

Plants were harvested at 8, 24, or 72 HAT. The plants were removed from treatment vials after 8 h. Roots were rinsed for 20 s in a stream of tap water, and plants were placed back in the hydroponic tank with herbicide-free, half-strength Hoagland solution. Samples harvested at 8 HAT were not returned to the tank. At all harvests, roots were blotted dry with paper towels, and shoots were separated from roots with shears. The samples were stored at -20 C for no more than 3 d before analysis.

Roots and shoots were minced separately. Samples were then homogenized (FSH 125, Fisher Scientific, 300 Industry Drive, Pittsburgh, PA 15275) for 30 s in 20 ml of acetone:water (90:10) in a 50 ml centrifuge tube. Samples were then sonicated for 1 h (Branson CPX8800H, Branson Ultrasonic, Danbury, CT 06810), centrifuged for 30 m at 4500 × g (Sorvall ST, Thermo Scientific, Waltham,

MA 02454), and the supernatant was transferred to separate tubes. A 2 ml aliquot of the supernatant was sampled, and radioactivity was quantified with LSC. Shoot and root residues were dried in a hood for 72 h and then combusted, and radioactivity was calculated with the previously mentioned methods. Extraction efficiency ranged from 85 to 97% and was comparable to levels reported in previous research (Mersie et al. 1995; Rouchaud et al. 1987).

Thin-layer chromatography (TLC) analysis was then conducted to evaluate metabolites. The supernatant was evaporated in a forced-air hood, and samples were resuspended in 100- μ l of acetone. The samples were spotted on 20 by 20 cm TLC plates that were developed to 16 cm in a glass chamber using benzene:acetic acid (95:5). Plates consisted of seven lanes that included six samples plus the parent herbicide diluted in acetone. The plates were air-dried, and metabolites were detected with a radiochromatogram scanner (BioScan System 200 Imaging Scanner, Bioscan, 4590 MacArthur Boulevard NW, Washington, DC 20007) connected to a computer equipped with Laura Chromatography Data Collection and Analysis Software[®] (LabLogic System, 1040 East Brandon Boulevard, Brandon, FL 33511). A metabolite of pronamide, 3,5-dichlorobenzoic acid (Santa Cruz Biotechnology, Dallas, TX 75220), was diluted in acetone, developed as previously described, and the retention factor (R_f) was identified with a fluorescence indicator.

Experimental Design and Data Analysis. The design for dose–response and PRE control experiments was a randomized complete block with five replications. Experiment 1 was conducted three times. Experiments 2, 3, and 4 were conducted twice. The design for absorption and metabolism experiments was a completely randomized design. Ten and five replications were used per run of the absorption and metabolism experiments, respectively. Three experimental runs were conducted for the root absorption experiment. The metabolism experiment was conducted twice.

Data were subjected to analysis of variance with the PROC GLM in SAS (v. 9.3, SAS Institute, Cary, NC 27513). Means were separated with Fisher's LSD test at $\alpha = 0.05$. For the dose–response experiments, data were analyzed with the PROC NLIN in SAS. Means were plotted on figures and regressed against the following three-parameter exponential-growth function model:

$$y = \beta_0 + \beta_1 * \exp(-\beta_2 * x) \quad [1]$$

where y is percent biomass of the nontreated, β_0 is the lower asymptote, β_1 is the upper asymptote, β_2 is the slope, and x is the pronamide application rate. The model was chosen that best described the plant responses to pronamide rates. The 95% confidence limits were used to compare pronamide rates that reduced biomass 50% from the nontreated. Experiment by treatment interactions were not detected, and results were therefore pooled over experimental runs.

Results and Discussion

Evaluation of Pronamide Resistance Levels. In Experiment 1, the pronamide rate required to reduce dry shoot biomass 50% from the nontreated (GR_{50}) from POST application at 4 WAT measured 0.7 and $>13.4 \text{ kg ha}^{-1}$ for the S and R biotypes, respectively (Figure 1; Table 1). In Experiment 2, the GR_{50} values for fresh shoot weights were similar between S biotypes from Georgia and North Carolina, averaging 1.3 kg ha^{-1} , respectively, while the GR_{50} for the R biotype was $>13.4 \text{ kg ha}^{-1}$ (Figure 2; Table 1). The resistance factor for this biotype to pronamide is >10 -fold higher than the S biotypes evaluated. These results support the supposition that this annual bluegrass biotype was resistant to POST pronamide applications.

There have been no reports of annual bluegrass resistance to pronamide. The only reported case of pronamide resistance was in a wild oat (*Avena fatua* L.) biotype from Oregon that was cross-resistant to acetyl-CoA carboxylase (ACCCase)-inhibitors. In that study,

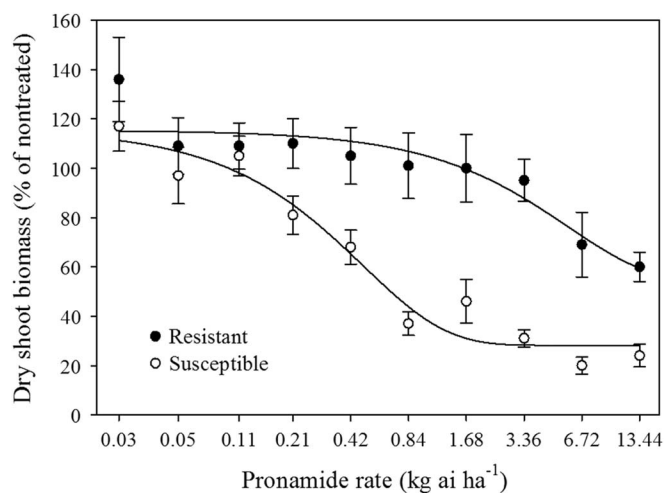


Figure 1. Dry shoot biomass for the resistant and susceptible annual bluegrass biotypes at 4 wk after POST pronamide treatments in three greenhouse experiments. Results were pooled over experimental runs.

Table 1. Regression analysis parameters for data presented in the figures.

Figure	Measurement	Biotype	GR ₅₀ ^a	95% CL for GR ₅₀	r ²	β ₀	β ₁	β ₂
			————— kg ai ha ⁻¹ —————					
1	Dry shoot biomass	Resistant	>13.44	4.4 to >13.44	0.10	94.36	34.71	5.83
		Susceptible-GA	0.7	0.5 to 0.9	0.57	28.91	87.41	2.12
2	Fresh shoot biomass	Resistant	>13.44	4.2 to >13.44	0.25	56.98	46.75	0.62
		Susceptible-GA	0.9	0.7 to 1.4	0.60	26.77	64.97	1.11
		Susceptible-NC	1.6	1.1 to 2.2	0.59	27.43	85.27	0.86

^aThe equation for the three-parameter exponential decay is $y = \beta_0 + \beta_1 * (\exp(-\beta_2 * x))$, where y is percent biomass of the nontreated, β_0 is lower asymptote, β_1 is the upper asymptote, β_2 is the slope, and x is pronamide application rate.

Seefeldt et al. (1994) reported that pronamide at 0.28 kg ha⁻¹ reduced the height of the R biotype 20% from the nontreated, whereas other biotypes had greater than 80% height reductions. It was surmised that the wild oat biotype had low levels of resistance to pronamide applications.

In Experiments 3 and 4, the efficacy of pronamide for PRE control of the R and S biotype from Georgia were evaluated. Treatment by biotype interactions were detected for plant count reductions relative to the nontreated (delineated as control) at 6 WAT. Indaziflam and both pronamide rates controlled the two biotypes >92% at 6 WAT (Table 2). Prodiamine controlled the S biotype 92% and 76% in Experiments 3 and 4, respectively, but the R biotype was controlled <17% in both experiments. Dithiopyr controlled the R and S biotypes 6 and 95% in Experiment 4, respectively.

The R biotype is resistant to PRE applications of dithiopyr and prodiamine but not pronamide. The susceptibility to PRE pronamide treatments was

also found in a prodiamine-resistant annual bluegrass biotype from North Carolina. Isgrigg et al. (2002) reported up to 105-fold resistance to prodiamine in annual bluegrass, but pronamide provided >93% PRE control of the R and S biotypes evaluated. Similar control levels were reported for pronamide in DNA-resistant biotypes of goosegrass from South Carolina and green foxtail from Canada (Delyé et al. 2004; Vaughn et al. 1987). Smeda et al. (1992) reported a green foxtail biotype was resistant to the DNA herbicides oryzalin, pendimethalin, and trifluralin, but was controlled by pronamide. PRE control of DNA-resistant annual bluegrass is attributed to the differences in the mechanisms of action of these mitotic inhibitors (Vaughan and Vaughn 1987; Vaughn and Lehnen 1991).

Annual bluegrass with high levels (>100-fold) of resistance to prodiamine has shown differential tolerance levels to other mitotic inhibitors. In North Carolina, it was noted that pendimethalin controlled a prodiamine-resistant annual bluegrass biotype equivalent to an S biotype. Differential levels of susceptibility to mitotic inhibitors were also found in annual bluegrass from Tennessee. Cutulle et al. (2009) reported the degree of annual bluegrass resistance to prodiamine was approximately 10-fold greater than dithiopyr and pendimethalin. Despite resistance to POST applications, pronamide could provide effective PRE control of this annual bluegrass biotype in our trial. Pronamide controls tillered annual bluegrass in late winter, but POST applications to seedling annual bluegrass in fall often provide more consistent control (Dow AgroSciences 2013; Johnson 1975). Efficacy of pronamide for controlling this biotype at various growth stages warrants further investigation.

The resistance to both prodiamine and pronamide at PRE and POST timings, respectively, is a novel finding in an annual grassy weed. There could be an association with resistance between these mechanisms of action that are dependent on the growth stage of

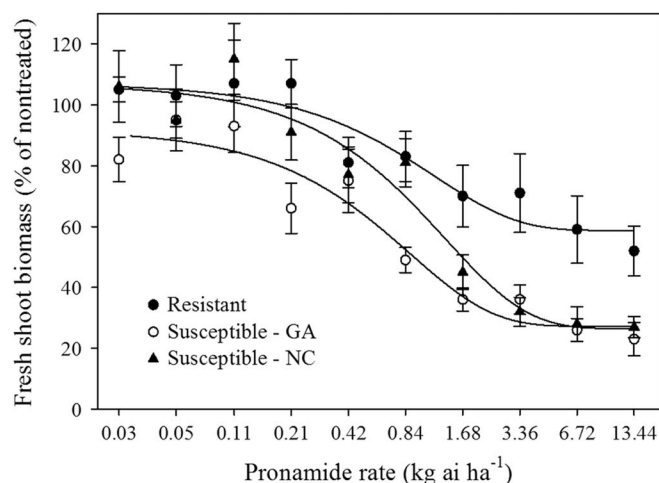


Figure 2. Fresh shoot biomass for the resistant and susceptible annual bluegrass biotypes at 4 wk after POST pronamide treatments in two greenhouse experiments. Results were pooled over experimental runs.

Table 2. PRE control of two annual bluegrass biotypes at 6 wk after herbicide treatments in two greenhouse experiments with results pooled over two runs conducted for each experiment.

Experiment	Herbicide	Rate (kg ai ha ⁻¹)	Control ^a	
			Resistant	Susceptible
			%	
3	Indaziflam	0.05	100	100
	Proflamifam	1.68	16	92
	Pronamide	0.56	100	100
		1.68	100	100
LSD _{0.05}			9	
4	Dithiopyr	0.42	6	95
	Proflamifam	1.68	0	76
	Pronamide	0.56	93	97
		1.68	98	98
LSD _{0.05}			18	

^a Plants were counted 6 WAT. Control was calculated as a percent reduction from the nontreated. Plant counts for the nontreated averaged 9 (± 1.7 SE) and 6 (± 1.5 SE) per pot for the R and S biotypes in Experiment 3, respectively, and 15 (± 1.5 SE) and 25 (± 2.7 SE) in Experiment 4, respectively.

the plant. For example, mutated tubulin may have reduced binding affinity with proflamifam that precludes PRE control at labeled use rates in turfgrass. The microtubule-associated proteins (MAPs) in this biotype could also have differential levels of susceptibility to pronamide at germination and at mature (3- to 5-tiller) growth stages. Overproduction of the target-site enzyme in mature plants has previously conferred resistance to ACCase inhibitors, glyphosate, and PPO inhibitors (Bradley et al. 2001; Pline-Srnic 2006; Warabi et al. 2001). Perhaps there is an overproduction of MAPs relative to S biotypes that would require higher pronamide rates to effectively inhibit mitosis. Further research is required to investigate the genetics and physiology of this annual bluegrass biotype to better understand the

differential tolerance levels to pronamide at various growth stages.

Absorption and Translocation of Radioactivity following [¹⁴C]Pronamide Applications. The R biotype absorbed 32% less radioactivity from [¹⁴C] pronamide than the S biotype at 72 HAT (Table 3). The specific radioactivity levels in roots (Bq g⁻¹) were similar between biotypes, but the R biotype accumulated 39% less radioactivity in shoots than the S biotype. Reduced levels of root absorption have been attributed to annual bluegrass resistance to atrazine in the absence of a mutated D1 protein (Svyantek et al. 2016). Root absorption levels also contribute to the selectivity of herbicides used for annual bluegrass control and the susceptibility of

Table 3. Absorption of [¹⁴C]pronamide, translocation of radioactivity, and specific radioactivity levels after 72 h in resistant and susceptible biotypes of annual bluegrass in three laboratory experiments with results pooled over experimental runs.

Biotype	Translocation % of ¹⁴ C absorbed	Absorption	Specific radioactivity ^a	
			Roots ^b	Shoots ^b
			Bq g ⁻¹	
Resistant	70	538	662	520
Susceptible	80	796	691	845
LSD _{0.05}	4	128	NS	155

NS, not significantly different according to Fisher's LSD test at $\alpha = 0.05$.

^a Specific radioactivity was quantified by dividing the radioactivity recovered in roots or shoots by the dry biomass of the roots or shoots. Absorption was calculated by dividing the sum of radioactivity in roots and shoots by the sum of the dry weight of roots and shoots.

^b The biomass of the roots and shoots measured 25 (± 2.8 SEM) mg and 85 (± 6.2 SEM) mg for the R biotype, and 38 (± 5.8 SEM) mg and 115 (± 12.8 SEM) mg for the S biotype respectively.

turfgrass species to injury from applications (Lycan and Hart 2006; McCullough et al. 2016; Singh et al. 2015; Yu and McCullough 2016; Yu et al. 2013).

The majority of the absorbed radioactivity from [^{14}C]pronamide was translocated to shoots in both biotypes, similar to previous reports in common amaranth, oat (*Avena sativa* L.) quackgrass [*Elymus repens* (L.) Gould], and chicory (Carlson et al. 1975; Mersie 1995; Table 3). However, the R biotype of annual bluegrass translocated 10% less of the total absorbed radioactivity as compared with the S biotype (70 vs. 80%). Differential levels of translocation have been previously reported between species susceptible and tolerant to pronamide. Mersie (1995) found 74% and 55% of the absorbed radioactivity from root-applied [^{14}C]pronamide was translocated to shoots after 72 h in a susceptible species, common amaranth, and a tolerant crop, chicory, respectively. Reduced levels of translocation after foliar absorption have also been associated with resistance to other herbicides, including glyphosate and paraquat (Shaner 2009; Yu et al. 2007).

Reduced absorption and translocation contribute to the tolerance of the R biotype to POST applications but do not explain the susceptibility to PRE treatments. The pronamide concentrations in soil were likely high enough to provide PRE control of the R biotype immediately after germination. Soil placement of pronamide is necessary to maximize efficacy, due to limited foliar uptake in mature weeds (Carlson et al. 1975; Dow AgroSciences 2013). However, seedling annual bluegrass may absorb pronamide through treated soil in the coleoptile that could enhance efficacy for PRE control of biotypes with resistance to POST applications.

Metabolism of [^{14}C]Pronamide. The R_f of [^{14}C]pronamide was identified at 0.69, and three metabolites were detected at 0.1, 0.4, and 0.9 in both annual bluegrass biotypes (Figure 3). These R_f values are consistent with previous research on pronamide metabolism in common amaranth, lettuce, and chicory, using the same chromatography methods (Mersie 1995; Rouchaud et al. 1987). The metabolite at R_f 0.1 was identified as 3,5-dichlorobenzoic acid through co-chromatography with the standard, similar to previous research (Rouchaud et al. 1987). Rouchaud et al. (1987) identified the metabolite at R_f 0.4 as the keto-derivative of pronamide, but the reference standard was unavailable for the work presented herein.

Harvest by biotype interactions were not detected for metabolism, and results were therefore pooled

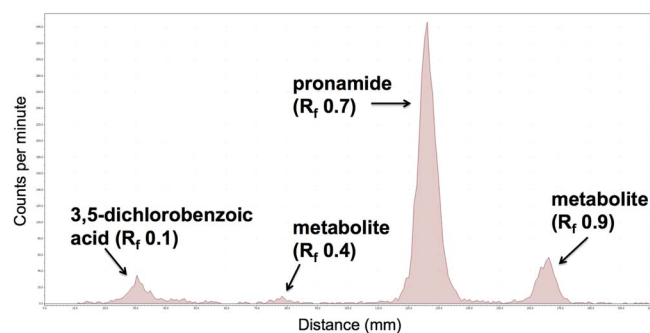


Figure 3. Radiochromatogram scan from a TLC plate image of pronamide metabolites from the R biotype at 72 HAT.

over main effects (Table 4). There were no meaningful differences detected in pronamide degradation from 8 to 72 HAT in the whole plant (roots + shoots). However, pronamide levels in roots declined over time from 34 to 13% of the total ^{14}C extracted from 8 to 24 HAT. Conversely, pronamide levels increased in shoots from 48 to 76% of the total ^{14}C from 8 to 24 HAT. Pronamide levels in shoots were similar at 24 and 72 HAT. The degradation levels are similar to previous research in common amaranth and witloof chicory, in which less than 20% of the absorbed [^{14}C]pronamide was metabolized after 48 h (Rouchaud et al. 1987).

The total levels of pronamide identified in the whole plant (roots + shoots) were similar among biotypes, averaging 84% of the extracted radioactivity (Table 4). The R biotype accumulated 7% less total pronamide in shoots (60 vs. 67%) and retained 5% more in roots (23 vs. 18%) as compared with the S biotype. Although these biotypes have comparable degradation rates of pronamide, the differences in herbicide distribution to shoots may be associated with the tolerance levels to POST applications.

The role of degradation of pronamide in plants has been shown to be inconsequential for selectivity. In previous research, common amaranth had similar metabolism to a tolerant species, witloof chicory (Mersie 1995). Alfalfa (*Medicago sativa* L.) has also shown slow metabolism of pronamide that was comparable to susceptible species (Yih and Swithenbank 1971). Reductions in pronamide efficacy have been associated with enhanced soil degradation rather than plant metabolism. In Australia, researchers noted sequential applications of pronamide were less effective in controlling rigid ryegrass (*Lolium rigidum* Gaudin) as compared with the initial treatment (Hole and Powles 1997). Reduced efficacy was attributed to enhanced herbicide degradation in soils after the initial treatment rather than the

Table 4. Metabolism of [¹⁴C]pronamide by two annual bluegrass biotypes in laboratory experiments with results pooled over two experimental runs.

Biotype	Roots				Shoots				Whole plant (roots + shoots)			
	Polar metabolites (R _f 0.1 + 0.4)		Nonpolar metabolite (R _f 0.9)		Polar metabolites (R _f 0.1 + 0.4)		Nonpolar metabolite (R _f 0.9)		Polar metabolites (R _f 0.1 + 0.4)		Nonpolar metabolite (R _f 0.9)	
	Parent (R _f 0.7)	Parent (R _f 0.7)	Parent (R _f 0.7)	Parent (R _f 0.7)	Parent (R _f 0.7)	Parent (R _f 0.7)	Parent (R _f 0.7)	Parent (R _f 0.7)	Parent (R _f 0.7)	Parent (R _f 0.7)	Parent (R _f 0.7)	Parent (R _f 0.7)
Resistant	4	23	3	60	4	4	6	83	8	8	9	9
Susceptible	2	18	3	67	4	4	6	85	6	6	9	9
LSD _{0.05}	NS	5	NS	7	NS	NS	NS	NS	2	2	NS	NS
HAT	5	34	6	48	2	2	5	82	7	7	11	11
24	1	13	2	76	4	4	4	89	5	5	6	6
72	3	14	1	67	6	6	9	81	9	9	10	10
LSD _{0.05}	2	6	2	8	2	2	3	5	2	2	3	3

NS, not significantly different at the 0.05 probability level.

pronamide application strategy evaluated. In the United Kingdom, degradation of pronamide was increased greater than fourfold in pretreated soil when compared with soil that received only one application (Walker and Welch 1991).

Increased pronamide metabolism was not detected in the R biotype relative to the S biotype. The S biotype translocated more pronamide to shoots than the R biotype. Conversely, the R biotype had more retention of pronamide in roots than the S biotype. Root metabolism of pronamide was limited (<7%) in both biotypes, suggesting the parent herbicide is moved to shoots within 24 h. Although pronamide may inhibit root formation of susceptible species, the data presented herein suggest that the herbicidal effects in annual bluegrass are concentrated in shoots.

Pronamide Resistance in Annual Bluegrass.

Annual bluegrass resistance to PRE and POST herbicides has risen exponentially in turfgrass. Currently, pronamide is used for controlling annual bluegrass resistant to ALS inhibitors, glyphosate, triazines, and DNA herbicides. Reduced absorption and translocation is a mechanism of resistance to POST pronamide applications in annual bluegrass. Biotypes with similar mechanisms for resistance to POST applications could still be susceptible to PRE pronamide treatments. The differential levels of control from PRE and POST applications suggest that resistance is not conferred with an altered target site in this biotype. The influence of absorption and translocation levels of pronamide is probably negligible for controlling seedlings as compared with mature annual bluegrass. Further research is needed to determine the relationship of annual bluegrass growth stage with pronamide resistance.

Since pronamide resistance has not been reported, these findings illustrate the diversity of mechanisms that could contribute to resistance in annual bluegrass. Turf managers must understand the potential for resistance development, strategies for controlling resistant biotypes, and methods for delaying resistance in annual bluegrass populations. Practitioners will need to incorporate integrated strategies that include modifications in cultural practices to control annual bluegrass, along with herbicide rotation or tank mixtures with multiple modes of action for sustainable management.

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